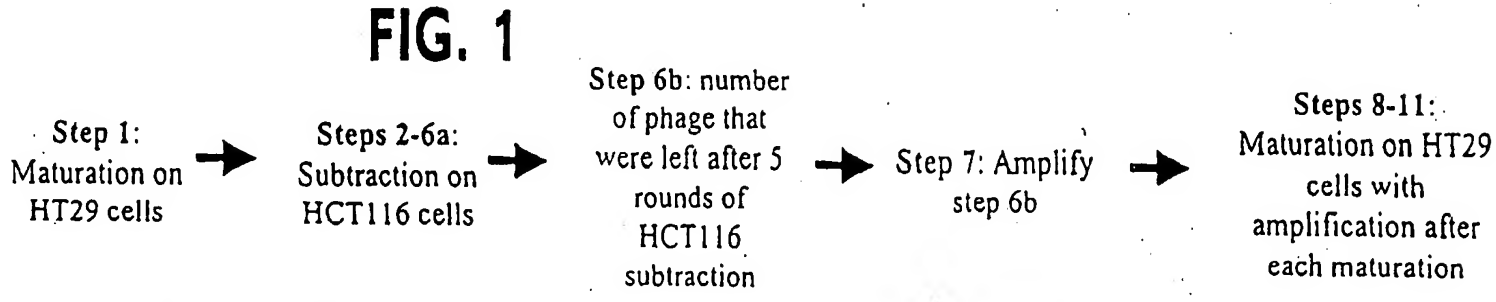
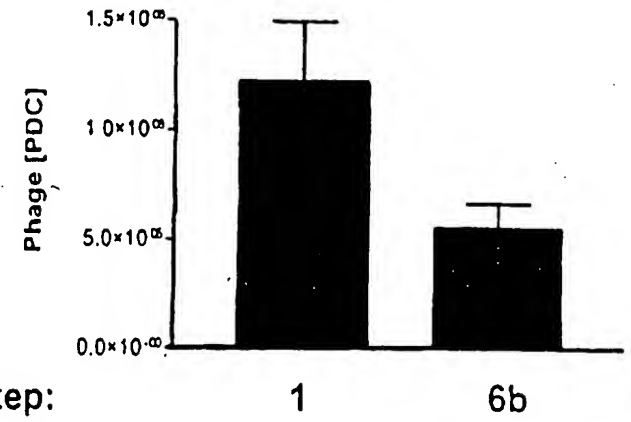
FIG. 1

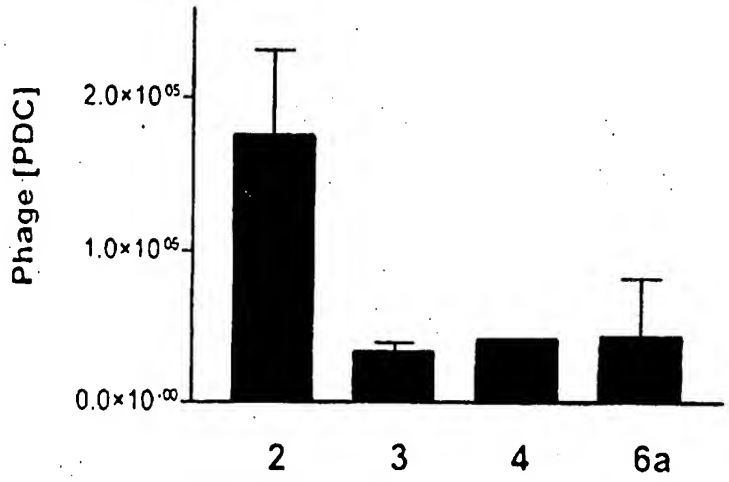
A.



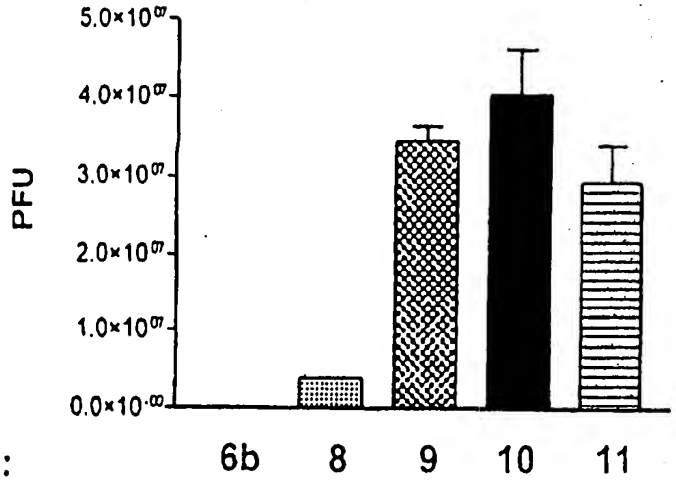
B.



C.

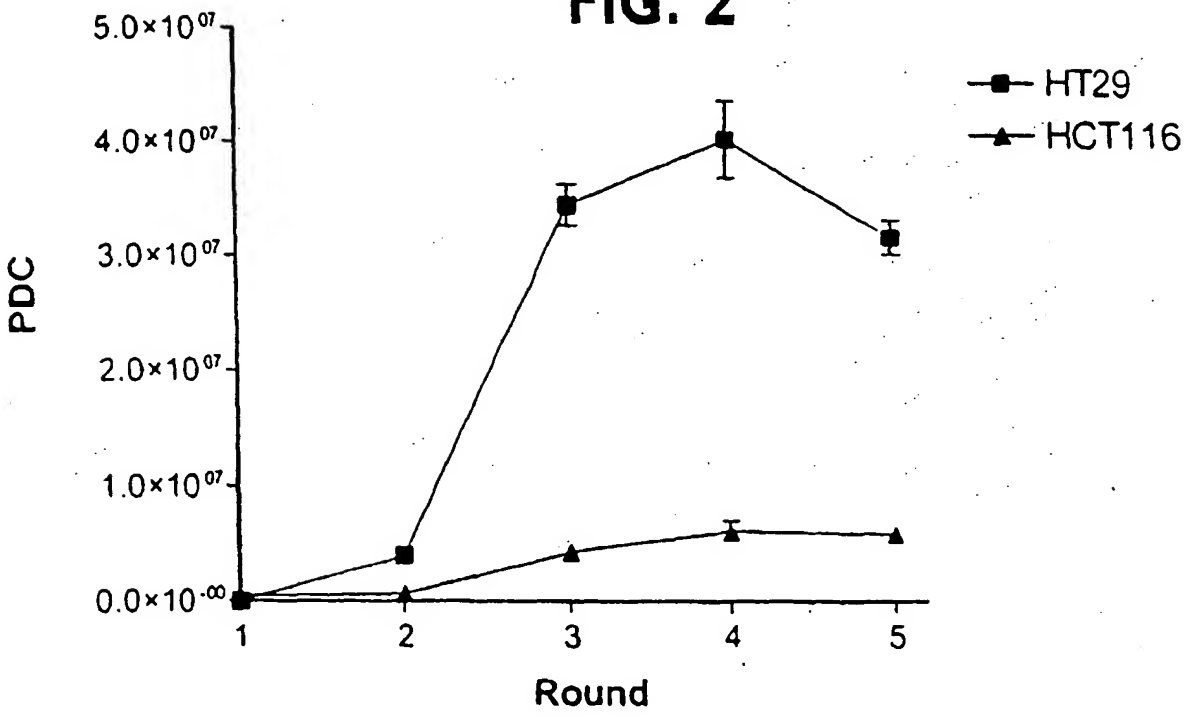


D.



Maturation of a phage pool that binds selectively to HT29 cells. A. Number of phage bound PCR to HT29 cells before and after subtraction from HCT116 cells as described in Materials and Methods. B. Number of phage bound to HCT116 cells after each round of subtraction. C. Maturation of phage library on HT29 cells. D. Schematic of phage maturation procedure. The phage titer was quantified by real time PCR.

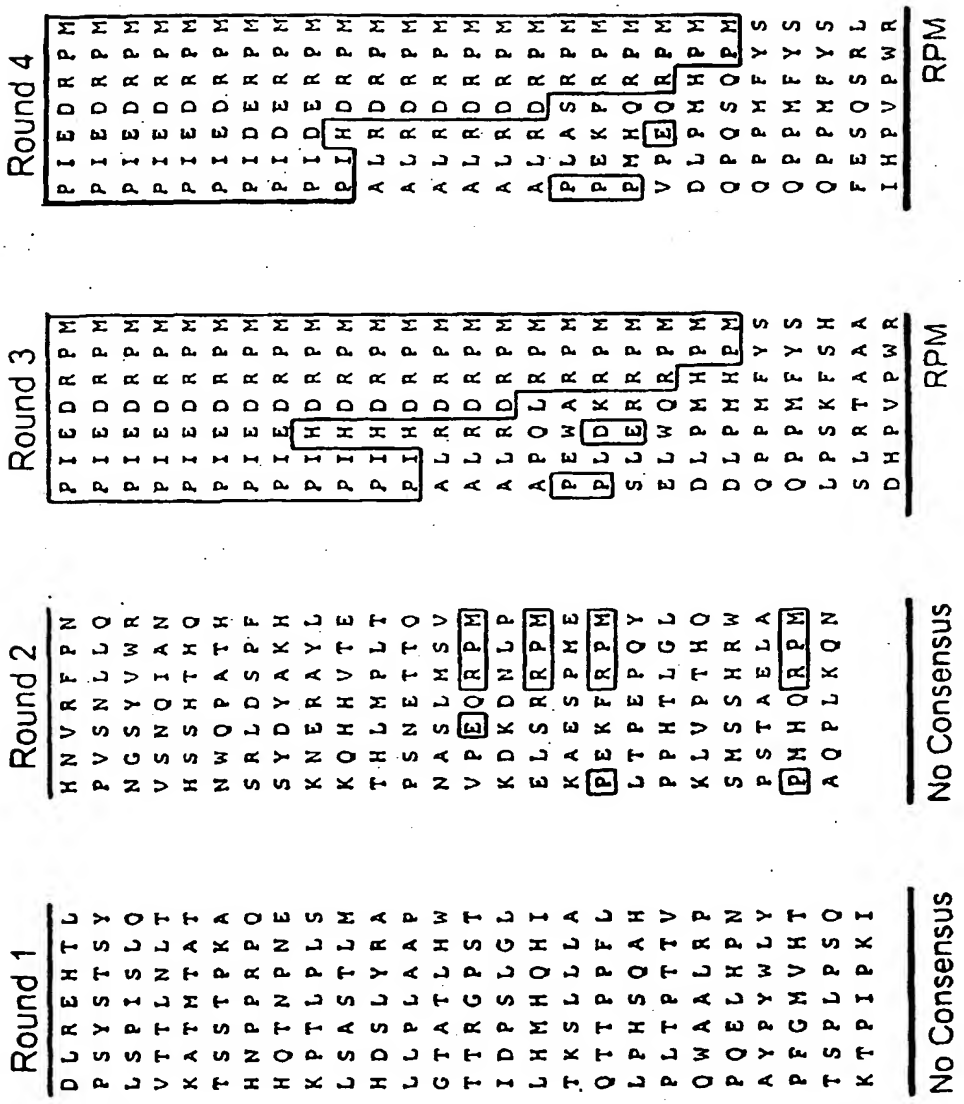
FIG. 2



The HT29 mature phage pool is selective for HT29 cellsthe amplified phage pool generated from each round of maturation. Phage remaining bound were quantified by real time PCR.

HT29 or HCT116 cells were incubated with 10^{10} pfu from

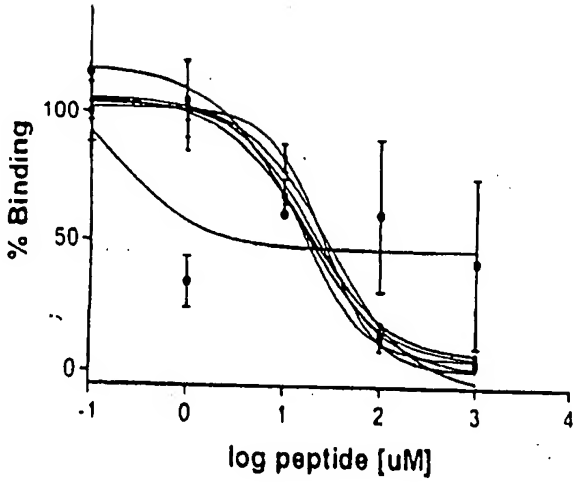
FIG. 3



RPM evolved by maturation on HT29 cells. Sequencing of phage from each round of maturation on HT29 cells was performed as described in Materials and Methods.

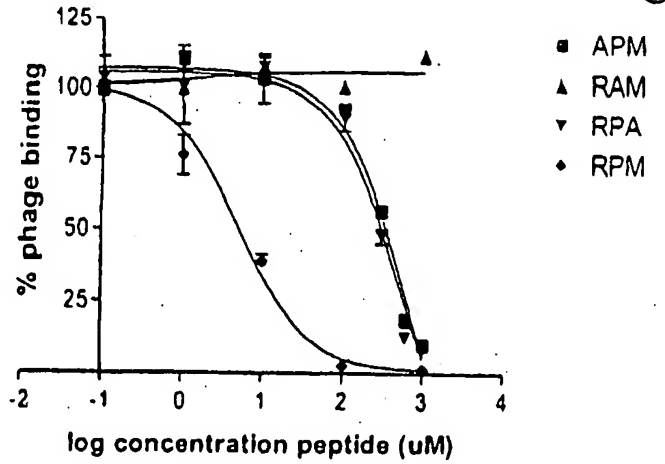
FIG. 4

A.



Binding to HT29 cells is dependent on the three amino acids, RPM, and their position within the peptide. A. HT29 cells were incubated with the 10^{10} PFU of indicated phage and increasing log concentrations of CPIEDRPMC peptide. B. As above, HT29 cells were incubated with RPM phage and increasing log concentrations of peptides with alanine mutations in the RPM sequence. C. HT29 cells were incubated with the indicated concentration of either CPIEDRPMC (RPM) or CPIRPMEDC (RPM middle) peptide and 10^{10} pfu of RPM phage. In all panels, the number of phage remaining bound to the cells was quantified by real time PCR.

B.



C.

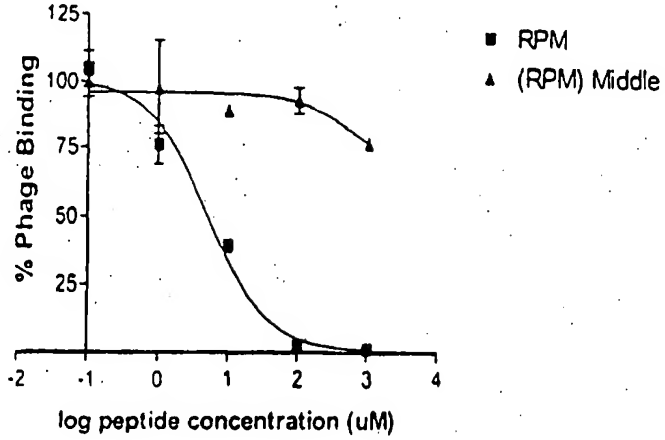
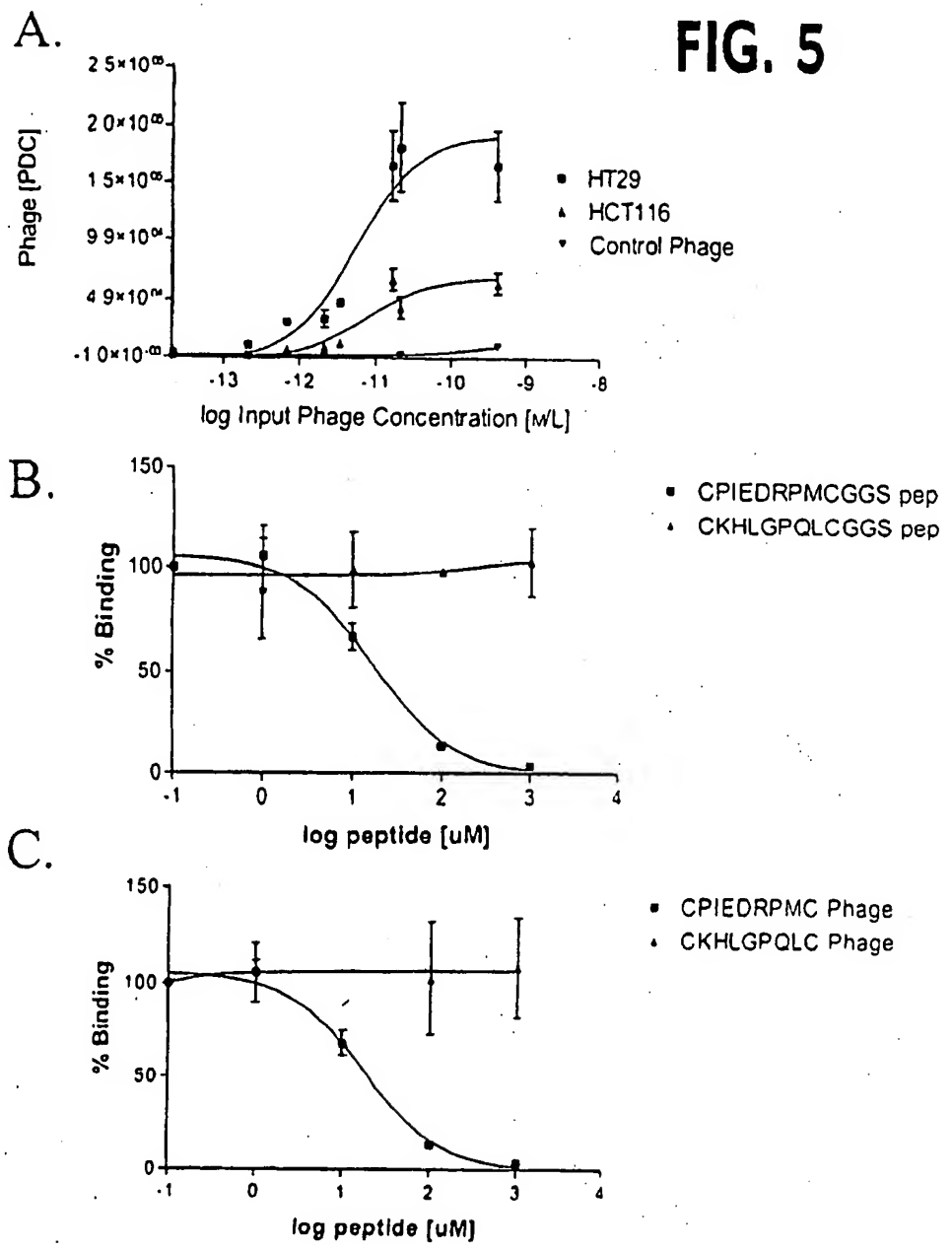


FIG. 5



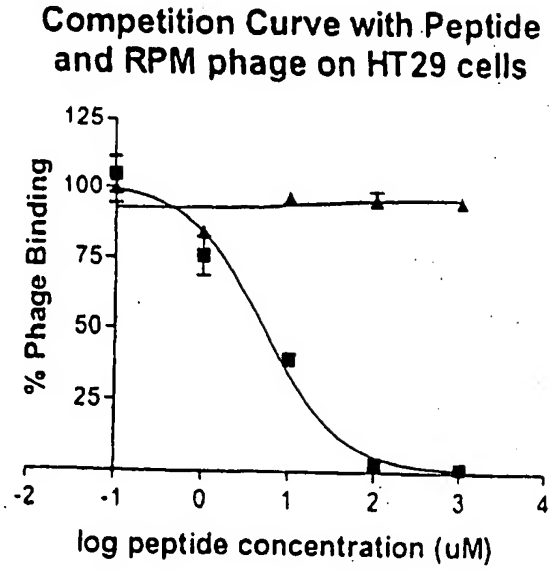
Phage displaying the RPM motif bind selectively and specifically to HT29 cells. A. HT29 or HCT116 cells were incubated with phage bearing the peptide CPIEDRPMC or phage that did not bind either cell line (Control Phage). The number of phage remaining bound to the cells was quantified by real time PCR B. HT29 cells were incubated with the 10^{10} PFU of CPIEDRPMC phage and the indicated concentrations of either specific or nonspecific peptide. The number of phage remaining bound to the cells was quantified by real time PCR. C. HT29 cells were incubated with 10^{10} PFU of either CPIEDRPMC or CKHLGPQLC phage and the indicated concentration of specific peptide. Phage remaining bound to the cells were quantified by real time PCR.

FIG. 6

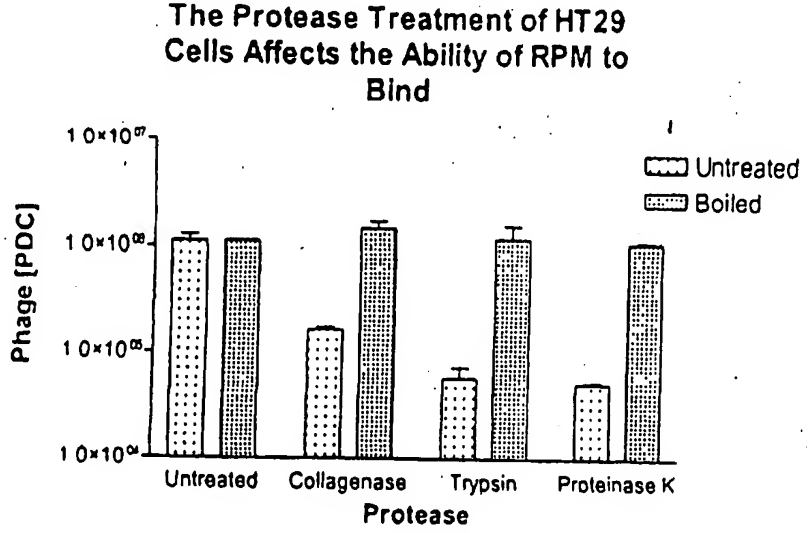
RPM peptide binds selectively to HT29 cells. A. A well of HT29, HCT116, or a mixture of 50% HT29 and HCT116 cells were incubated with FITC-RPM (red), fixed then incubated with a monoclonal antibody to p53 (green). Slides were analyzed by confocal microscopy. Nuclei are colored blue. B. Competition assay. HT29 cells were incubated with FITC-RPM in the presence of excess unlabeled specific or unlabeled non-specific competitor then analyzed by confocal microscopy.

FIG. 7

A.



B.



C. MTT viability assay

RPM peptide binds to a HT29 protein. A. HT29 cells were incubated with the 10^{10} PFU of RPM phage and increasing log concentrations of RPM peptide containing all D amino acids. B. HT29 cells were incubated for 15 minutes with collagenase, 5 minutes with Trypsin, or 1 minute with Proteinase K. As a control, the proteases were boiled for 15 minutes and then cells were incubated with the boiled proteases as above. After incubation with the respective protease, cells were incubated with 1010 pfu of RPM phage. C. The effect of protease incubation on HT29 viability was determined using an MTT assay. Cells were treated with proteases as in A. After treatment, MTT was added to a final concentration of 250 ug/mL and incubated for 45 minutes at 37 C. Following incubation with MTT, incorporation of the dye by the cells was assayed by plate reader set to absorb at 570nm. In A and B, the number of phage remaining bound were quantified by real time PCR.

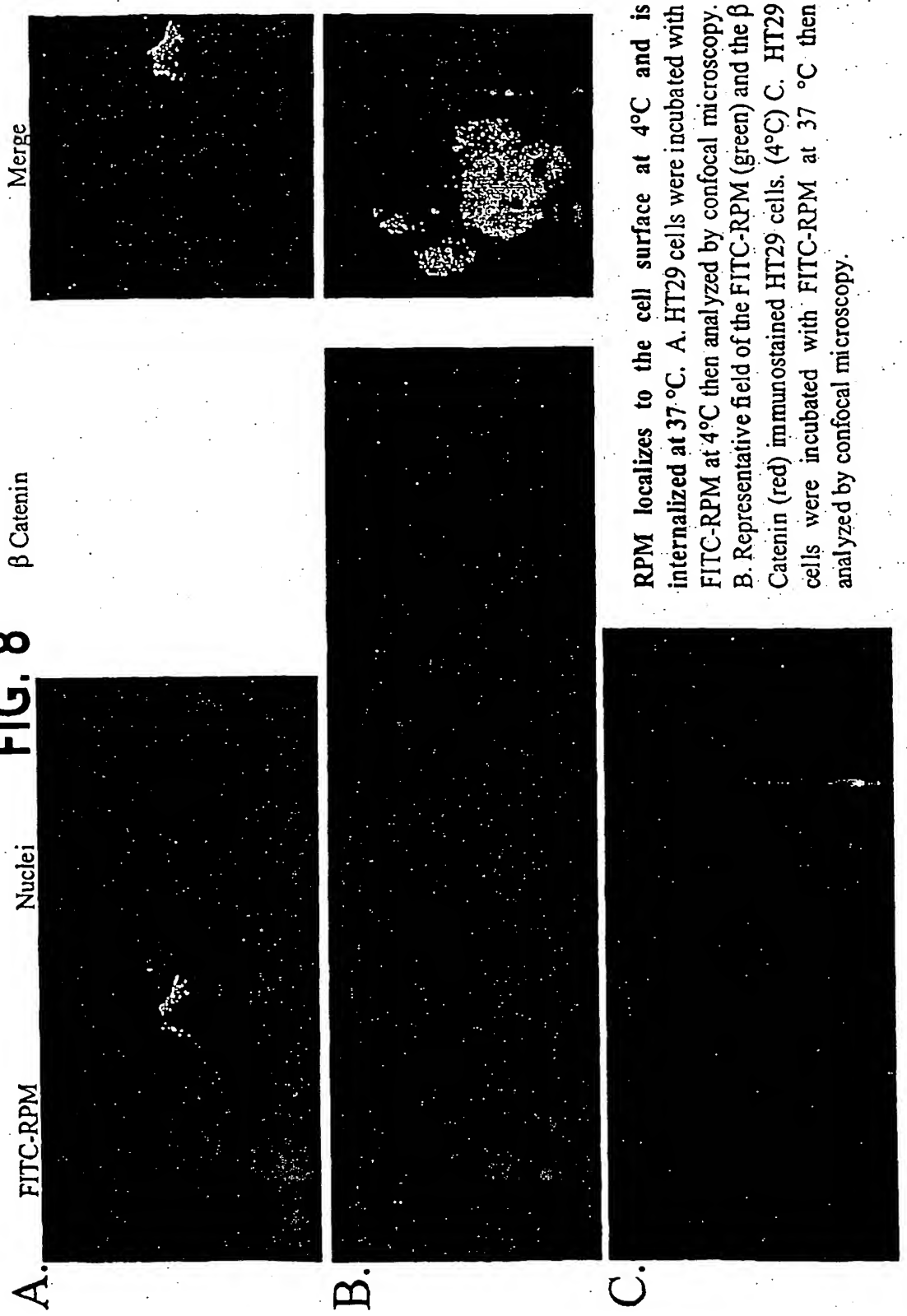
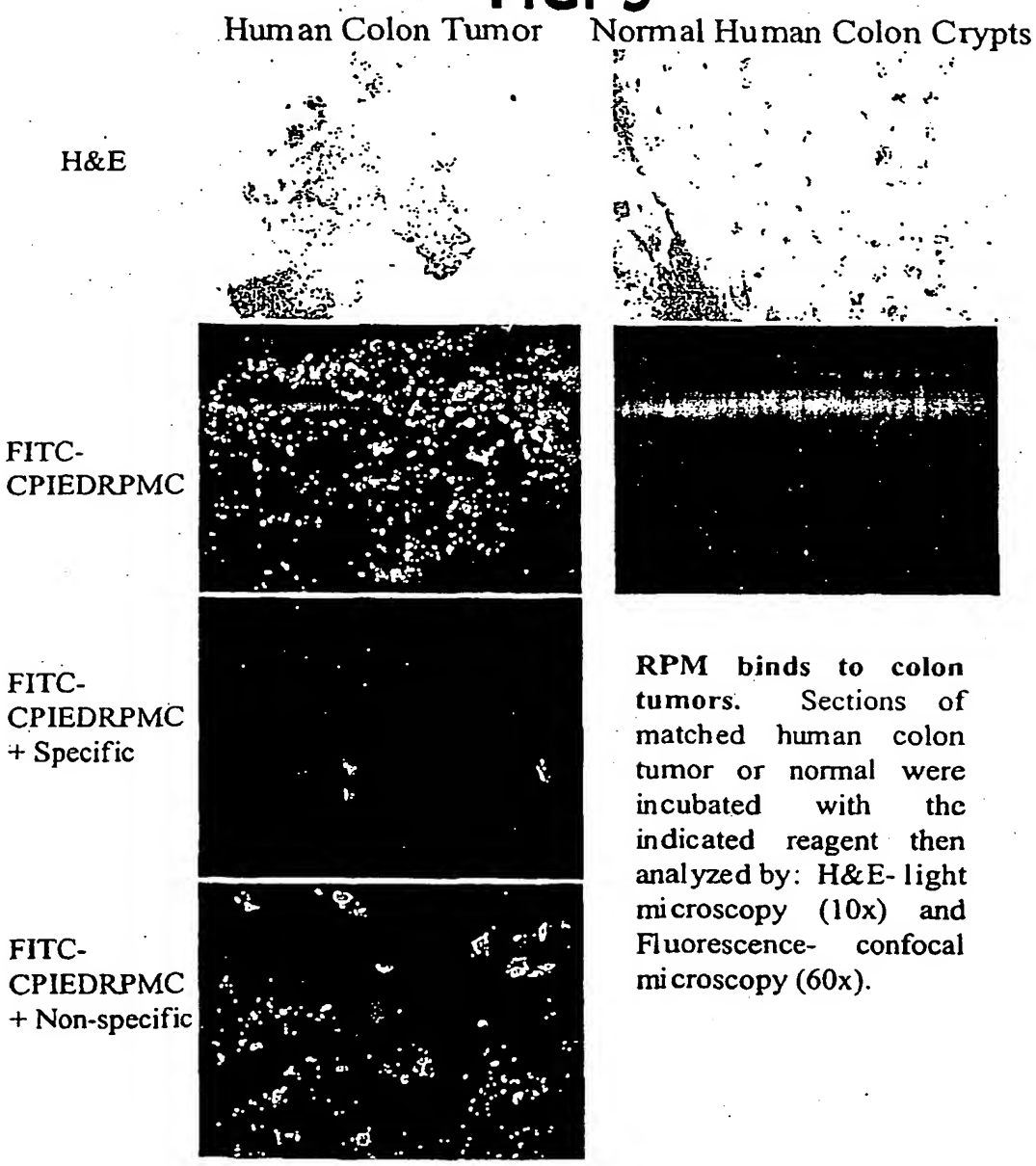


FIG. 8

RPM localizes to the cell surface at 4°C and is internalized at 37 °C. A. HT29 cells were incubated with FITC-RPM at 4°C then analyzed by confocal microscopy. B. Representative field of the FITC-RPM (green) and the β Catenin (red) immunostained HT29 cells. (4°C) C. HT29 cells were incubated with FITC-RPM at 37 °C then analyzed by confocal microscopy.

FIG. 9



RPM binds to colon tumors. Sections of matched human colon tumor or normal were incubated with the indicated reagent then analyzed by: H&E- light microscopy (10x) and Fluorescence- confocal microscopy (60x).

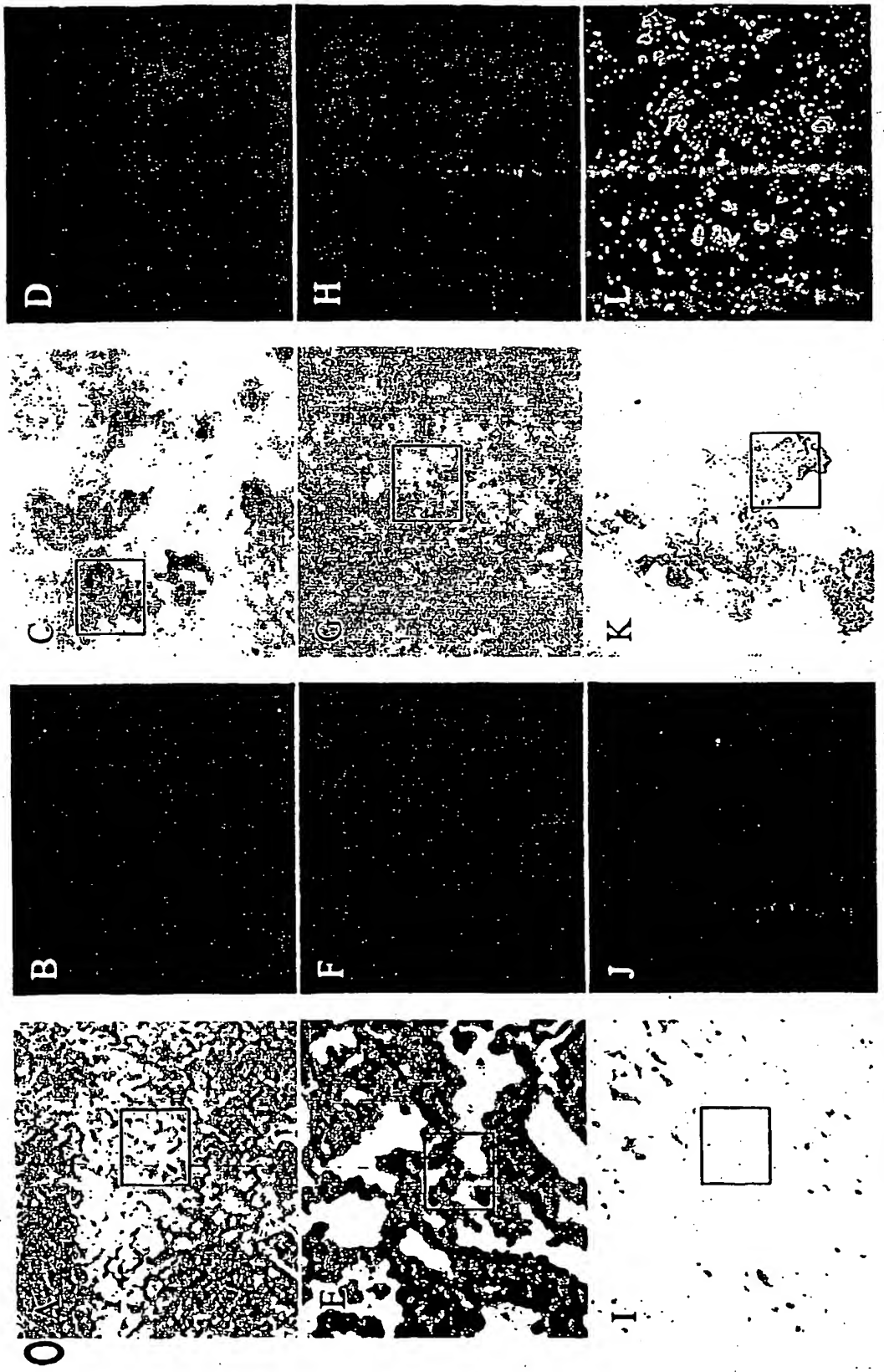
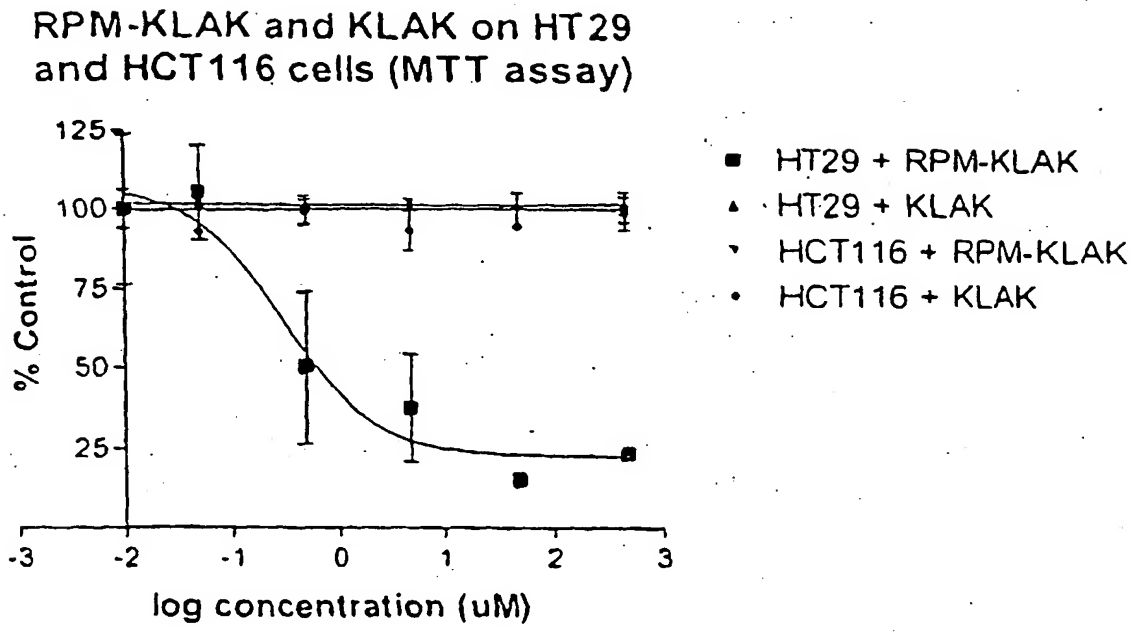


FIG. 10

RPM does not bind to normal lung, liver or stomach or to liver or lung cancer. A and B. Grossly univolved liver. C and D. Liver sarcoma. E and F. Grossly univolved lung. G and H. Lung sarcoma. I and J. Normal Stomach. K and L Colon Tumor. B,D,F,H, J, and L. Fluorescence microscopy of indicated tissues incubated with RPM-FITC and Topro-3. (60x). A,C,E,G,I, and K. H&E staining of the corresponding views (10x).

FIG. 11



RPM-KLAK kills HT29 cells. HT29 and HCT116 cells were incubated with increasing log concentrations of either RPM-KLAK or KLAK for 72 hours at 37°C. After incubation, cell viability was determined by MTT assay. The percentage viability was determined by dividing the absorbance units of a sample well by the absorbance units of the vehicle treated well.

FIG. 11 (Cont.)

Material and Method for selection and subtraction: An aliquot of the complete phage library from NEB was incubated with 2x105 cells (step 1). B. Phage that bound were eluted and incubated with the same number of HCT116 cells for a total of 5 incubations (steps 2-6). The phage that bound the HCT116 cells was eluted and the number of plaque forming units was determined by real time PCR. C. The number of phage that did not bind the HCT116 cells after five rounds of depletion was determined . The phage were amplified (step 8) then incubated with 2x 105 HT29 cells. Cells were washed to remove unbound phage and the bound phage was eluted. The number of phage bound was determined and the remaining eluate was amplifed. The amplified phage was used with the same number of HT29 cells and the process was repeated (steps 9-12) for a total of five rounds of maturation.